Poster Session 3 – Pharmacognosy

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Vasoactive agents from *Arnica montana*

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Preparations containing extracts from *Arnica montana* (Leopard's bane) are used for symptomatic relief from arthritis, local skin irritation and inflammation and bruising. Using a methanolic tincture prepared from Spanish *A. montana* flower heads, we have previously shown that sesquiterpene lactones from the plant extract were able to traverse human skin in-vitro, though at very small levels (Tekko et al 2003); here we report an in-vivo study showing that vasoactive agents are present in this tincture. A commercially available stock tincture of *A. montana* in ethanol–water (45:55, v/v) was obtained (Herbal Apothecary). A more concentrated solution in methanol–water (50:50, v/v) was prepared from this tincture to provide *Arnica* constituents at 10-times the initial concentration. Additionally, a sesquiterpene lactone extract was obtained from the stock tincture by evaporation under vacuum at ambient temperature to half its volume followed by extraction with methylene chloride–ethyl acetate (50:50, v/v) according to Leven & Willuhn (1987). This fraction was reconstituted into methanol–water (50:50, v/v) to give a 10-fold concentrated sesquiterpene lactone extract solution. Samples (15 μL) of the concentrated test solutions (tincture and sesquiterpene extracts) or the controls (methanol–water (50:50, v/v), betamethasone and nicotinic acid) were applied to duplicate occluded template areas on the ventral forearm of two subjects (with their informed consent). After 3 h, a quantitative assay of vasoactivity was obtained by examining change in the skin site's 1931 CIE chromaticity co-ordinates using a PR-650 Spectrascan Colorimeter (Photo Research Inc., Chatsworth, CA). This device gives a measure of any colour shift and was validated using betamethasone (vasoconstrictor, skin blanching) and nicotinic acid (vasodilator, erythema). After 3 h, vasodilation was clearly observed with the concentrated tincture and this was accompanied by a change in the measured chromaticity co-ordinates of the skin surface towards the red region of the chromaticity space of $13.5 \times 10^{-3} \pm 8 \times 10^{-3}$ (mean $\pm$ s.d., n = 4). This effect was similar to that of nicotinic acid 1%, w/v $12.7 \times 10^{-3} \pm 4.9 \times 10^{-3}$ in comparison with the blank solution which resulted in a shift of $-1.9 \times 10^{-3} \pm 2.3 \times 10^{-3}$. Moreover, the concentrated sesquiterpene lactone extract showed similar activity to the entire concentrated tincture with a similar shift in chromaticity co-ordinates of $11.7 \times 10^{-3} \pm 9 \times 10^{-3}$ (mean $\pm$ s.d., n = 4). This provides evidence to support our earlier suggestion that it is the sesquiterpene lactone fraction within *Arnica montana* that provides some of the activity reported for these preparations. Additionally, the rubefacient activity of the sesquiterpene lactone concentrate was still apparent on one subject up to 48 h post-application and so it can be concluded that these components from *Arnica* could have a profound vasodilatory effect if judiciously formulated.

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The flavonoidal constituents of *Arthrocnemum glaucum* (Del) and their antimicrobial activity

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The family *Chenopodiaceae* shows an interesting geographical distribution, which is determined by the fact that they are almost halophylic. Vegetables of this family generally contain 1-O-ferulyl-β-D-glucose (Rizk 1986). *Arthrocnemum* is a genus of this family, which is represented in Egypt by only one species (Tackholm 1974). *A. glaucum* (Del) is reported to contain isoquinoline alkaloids, which were identified as (+)-carnegin, (−)-1-methyl corypalline, (*R*-(+)-salsolidine and nigellimine. The investigation of the steroid content revealed the presence of fucosterol and two other C_{29} sterols. It was found from literature that the ethyl alcohol extract of the plant showed moderate molluscicidal activity against *Biomphalaria alexandrina* snails (Nazif et al 2000). The phytochemical screening of *A. glaucum* growing in Egypt revealed the presence of flavonoids, sterols, triterpenes, tannins and alkaloids. Nothing was reported about the flavonoid constituents of *A. glaucum* (Del), therefore this work deals with the study of the flavonoid constituents of the plant and studying the antimicrobial activity of total extracts and the isolated flavonoidal compounds. Plant materials (aerial parts) were collected from Borg-El Arab near Alexandria during Feb 2000 and March 2002, dried, powdered and extracted with petroleum ether and then with 70% methanol. The methanolic extract, after partitioning with chloroform and ethyl acetate, yielded crude extracts containing flavonoids. The chloroform extract was subjected to preparative PC (3 MM, 15% acetic acid), and the main flavonoidal band was cut and eluted with methanol. The eluted fraction was subjected to further purification using Sephadex LH-20 column using 90% methanol. The isolated flavonoidal compound was identified as apigenin. The ethyl acetate fraction was subjected to preparative PC (3 MM, 15% acetic acid). The flavonoidal bands were cut and subjected for further purification using Sephadex LH-20 column using 90% methanol. The study of the ethyl acetate fraction revealed the isolation of isorhamnetin 3-O-glucoside, isorhamnetin and ferulic acid. The identity of the isolated flavonoids were verified by TLC, PC, UV, NMR and FAB-MS in addition to acid hydrolysis of the flavonoidal glycoside and identification their sugar moieties. This is the first record of the flavonoids in *A. glaucum*. The antimicrobial activity of total extracts and the isolated flavonoidal compounds were carried out using pathogenic micro-organisms, namely, *Escherichia coli*(G−ve), *Bacillus subtilis* (G+ve), fungi (*Aspergillus niger*) and yeast (*Saccharomyces cerevisiae*). The ethyl acetate fraction showed high antimicrobial activity against G−ve bacteria (*E. coli*), yeast (*Saccharomyces cerevisiae*) and fungi (*Aspergillus niger*). The flavonoidal compound isorhamnetin 3-O-glucoside isolated from the ethyl acetate fraction showed high antimicrobial activity against G−ve bacteria (*E. coli*), while isorhamnetin and ferulic acid showed moderate antimicrobial activity against *E. coli* (G-ve) and *Bacillus subtilis* (G+ve). Three different concentrations (2, 4 and 6 mg mL^{-1}) of each extract and isolated compounds were used and the minimum inhibitory concentration was found to be 6 mg mL^{-1}.

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Activity of saponins from *Medicago* species against dermatophytes

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Saponin-rich extracts of *Medicago sativa* L., commonly known as Alfalfa or Lucerne, have been shown to display antifungal activity against a range of common fungi and plant pathogens but they have not been tested against dermatophytes and none of the individual saponins has been tested (Zehavi & Polacheck 1996). This study was carried out to determine the activity of extracts of other *Medicago* species and saponin glycosides extracted therefrom. Total saponins were separately obtained from roots and aerial parts of *Medicago sativa, M. murex, M. arabica* and *M. hybrida.* Four isolated glycosides of medicagenic acid, one of hederagenin and one of soyasapogenol were also available and all eight extracts and six compounds were tested against three species of dermatophytic fungi, *Microsporum gypseum* NCPF 236, *Trichpophyton interdigitale* NCPF 81 and *T. tonsurans* NPCF 236. Twenty microlitres of a standard fungal spore suspension 10^4 cfu were seeded in microwell plates containing 200 μL double-strength Saboraud dextrose agar and 200 μL of the extract or compound in 4% DMSO were added in a range of concentrations from 4 mg mL^{-1} to 0.0625 mg mL^{-1}. Each plate was incubated at 30°C for 24 h and three replicates were carried out for each substance, using miconazole 400 μg mL^{-1} as a positive control. The lowest concentration showing absence of fungal growth was recorded as MIC. Results are shown in Table 1. *T. tonsurans* appears to be the most sensitive of the dematophytes to the active compounds. Glycosides of medicagenic acid were the most active compounds, especially the 3-O-glucoside. TLC analysis (silica gel/EtOAc:AcOH:H2O 7:2:2 detection after spraying with anisaldehyde reagent) of the extracts showed no clear correlation between content of identifiable compounds and activity although many compounds present were not able to be identified.

Table 1 MIC values (mg mL^{-1}) for total saponins and individual saponins of *Medicago* spp.

Substance	*M.gypseum*	*T.inter-digitate*	*T. tonsurans*
M. sativa roots	0.5	1.0	< 0.0625
M. sativa aerial	0.5	1.0	< 0.0625
M. murex roots	0.125	0.125	< 0.0625
M. murex aerial	0.5	0.5	< 0.0625
M. arabica roots	0.125	0.125	< 0.0625
M. arabica aerial	0.125	0.125	< 0.0625
M.hybrida roots	2.0	0.5	0.25
M.hybrida aerial	2.0	2.0	0.5
MA 3-OGlu	< 0.0625	< 0.0625	< 0.0625
MA 3,28-OGlu	> 1.0	1.0	0.25
MA 3-Oglucose 28-ArRhXyl	1.0	0.5	0.25
MA 3-OgluA 28-ArRhXyl	> 1.0	> 1.0	> 1.0
H 3-OArGluAr	> 1.0	> 1.0	> 1.0
S 3-GluAGaRh	> 1.0	> 1.0	> 1.0

MA = medicagenic acid, H = hederagenin, S = soyasapogenol, Glu = glucose, GluA = glucuronic acid, Ar = arabinose, Rh = rhamnose, Xyl = xylose.

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Effect of methanolic extract of *Eclipta alba* L. on lipid peroxidation and free radical scavenging activity

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Oxidative stress that results from the increased generation of reactive oxygen species has been implicated in major human ailments like cardiovascular disease, cancer, neural disorders and in the process of aging. Hence, this study aimed to evaluate the in-vitro antioxidant activity of the methanolic extract of the whole plant of *Eclipta alba* (L.) Hassk. (Asteraceae). It has been widely used in India for the traditional treatment of liver disorders. The dried methanolic extract was evaluated for interaction with 1,1-diphenyl, 2-picryl hydrazyl (DPPH•) stable free radicals (Blois 1958), hydroxyl radical scavenging activity and inhibition of lipid peroxidation in-vitro. The dried powdered whole plant of *Eclipta alba* was extracted with methanol in a soxhlet extractor. The methanolic extract was evaporated to dryness under reduced pressure below 50°C. The antiradical scavenging potential of the extract was assessed by adding to the solution of DPPH an equal volume of the test extract dissolved in ethanol at various concentrations (10–400 μg mL^{-1}). Hydroxyl radical scavenging activity was evaluated using ascorbic acid, iron-EDTA model of •OH generating system. The formaldehyde formed during the oxidation of dimethyl sulphoxide by the Fe^{3+}-ascorbic acid system was used to detect hydroxyl radicals (Klein et al 1981). To the reaction mixture, test extracts were added at various concentrations (10–100 μg mL^{-1}) and formaldehyde formed was assayed spectrophotometrically. Mannitol was used as reference standard. The level of lipid peroxidation in the rat liver homogenate was measured in-vitro as thiobarbituric acid reactive substances (Ohkawa et al 1979). Malondialdehyde formed was quantified using a molar extinction coefficient of 1.56×10^{-5} M^{-1} cm^{-1} and expressed as U mg^{-1} of protein. The reference standard was α-tocopherol. The methanolic extract *Eclipta alba* showed significant ($P < 0.001$) interaction with stable free radical DPPH$^{\cdot}$ expressing scavenging activity in the concentration range of 10–400 μg mL^{-1} to the highest extent of 77.82%. Further, the oxidation of DMSO was significantly ($P < 0.001$) inhibited indicating •OH radical scavenging activity in the concentration range of 10–100 μg mL^{-1}. It exhibited 21.12% inhibition in the formaldehyde produced at a concentration of 100 μg mL^{-1}. The extract inhibited the lipid peroxidation induced in the rat liver homogenate significantly ($P < 0.001$) in the concentration range of 50–400 μg mL^{-1} in a dose-dependent manner. At a concentration of 400 μg mL^{-1} the extract exhibited 78.86% inhibition of lipid peroxidation. Thus the studies revealed that the methanolic extract of *Eclipta alba* exhibits significant in-vitro antioxidant and free radical scavenging activity. Further research to explore the antioxidant potential of this plant is required as it can be exploited in reducing free radical induced pathophysiological abnormalities.

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Alkaloids from two Nigerian *Crinum* species and their acetylcholinesterase inhibitory activity

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The bulbs of *Crinum jagus* (Thomps.) Dandy and *C. glaucum* A. Chev. (Amaryllidaceae) are used in traditional medicine in southern Nigeria for memory loss and other mental symptoms associated with ageing. Memory loss is associated with depressed levels of acetylcholine (ACh) and this can be reversed by the administration of inhibitors of acetylcholinesterase (AChE), an approach used therapeutically in the treatment of Alzheimer's disease. Methanol extracts of the bulbs of both species showed inhibition of AChE with the Ellman spectrophotometric test (Perry et al 2000) and this was shown to be associated with the alkaloids after standard acid-base fractionation. Using the in-situ bioautographic test method for enzyme inhibition and checking that no false positive effects were observed (Rhee et al 2001), a number of alkaloids were isolated and their structures determined by mass and NMR spectroscopy. Four alkaloids were isolated, all of which had been previously isolated from *Crinum* species. Their activity was quantified using the Ellman test and a range of concentrations so that the IC50 value could be calculated. The most active alkaloids hamayne (IC50 250 μM) and lycorine (IC50 450 μM) while other alkaloids were comparatively inactive, with haemanthamane giving 3% inhibition and crinamine giving 4.4% inhibition at 50 mg mL^{-1} (174 μM). These contrast with the positive control, physostigmine, which gave IC50 of 0.25 μM. Cholinesterase activity appears to be associated with the presence of two free hydroxy groups in this structural type of Amaryllidaceae alkaloid. This is the first report of alkaloids from *C. glaucum* and the AChE inhibitory activity of these compounds has not previously been reported.

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Profiling opium alkaloids in opium samples and poppy straw by capillary electrophoresis

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The most commonly used method of alkaloid separation from opium samples is gas liquid chromatography, but samples have to be derivatised and chromatography of some alkaloids is poor. High-performance liquid chromatography is an alternative, but the method lacks the resolution required for the separation of closely related alkaloids present in the samples. Capillary zone electrophoresis (CZE), because of its high resolving power, has the capacity to separate the alkaloids present in opium (Trenerry et al 1995). This work reports the separation and quantification of the main alkaloids found in opium samples and poppy straw using CZE. The separation of a standard set of alkaloids, along with an internal standard (dihydrocodeine) has been demonstrated for the main alkaloids (morphine, codeine, papaverine and thebaine (Dewick 1997)). Other alkaloids — narceine, noscapine, oripavine and reticuline — are included although present in smaller quantities. Apparatus consisted of a Hewlett Packard ^{3D}CE; fused silica capillary (50 μm internal diameter: effective length 65 cm), buffer system 50 mM di-sodium phosphate, 80 mM sodium dodecylsulphate (SDS) and 25% methanol at pH 2.5. Applied voltage was −30kV with detection at 200 nm. Opium samples from various locations were analysed for alkaloid content and results are shown in Table 1. Poppy straw samples (S1-S5 Tasmanian Alkaloids) (1 g) were sonicated for 30 min in a mixture of 15 mL methanol:1 mL glacial acetic acid, diluted and alkaloid content measured. Results are shown in Table 2. Distinct profiles of opium samples from different geographical locations, along with those for different poppy straws have been generated. The method has been extensively validated with a detection limit of 100 ng mL^{-1} for morphine. Quantitative scoring of CE peak areas

and retention times for the profiles may be used to authenticate and assess quality of the samples.

Table 1 Alkaloid content of opioid samples

Compound	Persian	Indian	Turkish	Yugoslavian
Papaverine	7.3	ND	3.8	34.6
Noscapine	77.2	60.7	63.9	126.7
Thebaine	34.8	13.8	5.2	33.0
Reticuline	11.8	16.0	11.2	15.0
Oripavine	1.7	ND	ND	0.3
Codeine	36.0	26.1	15.0	28.1
Morphine	101.6	105.8	184.8	116.8

Concentrations are in mg/g of opium; ND, not detected.

Table 2 Alkaloid content of poppy straw samples

Compound	S1	S2	S3	S4	S5
Papaverine	ND	ND	2.56	3.88	3.68
Narceine	ND	ND	ND	0.72	0.84
Noscapine	0.66	ND	5.02	ND	ND
Thebaine	0.63	11.54	0.85	1.26	1.02
Reticuline	0.59	0.58	0.82	0.79	0.76
Oripavine	ND	6.04	ND	ND	ND
Codeine	0.82	ND	1.49	1.53	1.09
Morphine	23.83	ND	18.79	13.13	16.82

Concentrations are in mg/g of poppy straw; ND, not detected.

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Bioassay guided fractionation of Malaysian plants for potential anti diabetic activity

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Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and is linked with low blood insulin levels or insensitivity of target organs to insulin (Maiti et al 2004). An increasing number of patients suffering from diabetes — 150 million people in 2000, but twice that number is estimated by WHO to suffer by 2025 (Perion et al 2003). Although many drugs and interventions are available to manage diabetes, in many instances these are expensive for developing countries and have adverse effects and plants could provide useful aids in therapy if their activity could be validated. The aim of this study is to investigate the potential anti diabetic properties of Malaysian local plants using in-vitro models and to isolate the active compounds responsible for the activity using bioassay guided fractionation techniques. Six plant species were dried, powdered and extracted with aqueous and organic solvents. All extracts were tested in the in-vitro α-amylase inhibition model (Sigma Aldrich 2003). The most active extract was the hexane extract of *Phyllanthus amarus*, which gave a significant inhibitory effect with 21% inhibition at $1\,mg\,mL^{-1}$ and 72.4% inhibition when $0.05\,mg\,mL^{-1}$ was pre-incubated with the enzyme. The study showed that the pre-incubation method had more inhibition effect than the non-pre-incubation method. The hexane extract of *Phyllanthus amarus* was fractionated by prep-TLC with mobile phase hex:$CHCl_3$:MeOH (25:73:2) — full length of plate and $CHCl_3$:MeOH (3:1) — half-length of the plate. Ten percent of the plate was sprayed with anisaldehyde reagent and heated to detect zones. Five fractions were collected and tested in the bioassay. Fraction D (Rf $\sim$ 0.18) showed the most activity with 79.9% inhibition. To isolate compounds in this fraction, more of the plant extract was applied to vacuum liquid chromatography. Fractions were collected using hexane, hex:$CHCl_3$ (80:20), hex:$CHCl_3$ (50:50), $CHCl_3$, $CHCl_3$:MeOH (50:50) and methanol. Fractions with the same TLC profile were pooled resulting in 6 major fractions. Each fraction was then compared on TLC with the active fraction D and PA4, PA5 and PA6 showed the same compounds present as fraction D. The three fractions were tested in the bioassay and showed 83%, 70% and 65% inhibition, respectively. Work is currently in progress to isolate novel α-amylase inhibitors from fraction PA5 using column chromatography.

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Antimicrobial and antioxidant aspects of *Commelina diffusa*, a Ghanaian plant used traditionally for wound healing

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Many plant species are used in traditional medicine for aiding and accelerating the complex process of wound healing. A battery of in-vitro models is required to cover all activity that may be relevant but antimicrobial and antioxidant properties are considered to be beneficial and have been used in studies on such plants (Mensah et al 2001). The whole aerial parts of *Commelina diffusa* Burn. F (Commelinaceae) have been used in Ashanti traditional medicine in Ghana to help heal wounds and so a methanol extract was prepared and 100 μL used to test for antibacterial (4 species) activity using the disk diffusion assay. Results are shown in Table 1. Antifungal activity (6 species) was tested using serial dilution in a 96-well plate to obtain the MIC as shown in Table 2 (Mensah et al 2000). Chlorampenicol (C) 1% w/v and clotrimazole (CL) 1% w/v were used as positive controls. Antioxidant effects were tested using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), which becomes purple on oxidation (Cuendet et al 1997). No significant antioxidant activity was observed but the extract showed some antibacterial activity and selective antifungal activity against *Trichophyton* spp., a common dermatophyte (see Table 2). The antimicrobial activity suggests that the use of the plant in wound healing may be based on an antiseptic role.

This work was sponsored by a Wellcome Tropical Development Research Grant.

Table 1 Zones of inhibition given by 100 μL methanol extract of *C. diffusa* against bacteria

StA	BS	PA	EC	CHLOR
13.5 ± 0.5	6.5 ± 0.5	10.0 ± 0.0	11.0 ± 0.5	15.5 ± 0.5

Diameter is in mm; mean $\pm$ s.e.m., n = 3. StA, *Staphylococcus aureus*; BS *Bacillus subtilis*; PA, *Pseudomonas aeruginosa*; EC, *Escherriccia coli* (all from KNUST culture collection); CHLOR, chloramphenicol 1% w/v.

Table 2 MIC values given by methanol extract of *C. diffusa* against fungi

Ti	Tt	Mg	Ep	Sc	Ca	CLT
250	500	> 1000	> 1000	> 1000	> 1000	31

MIC is in $\mu g\,mL^{-1}$. Ti, *Trichophyton interdigitale*; Tt, *T. tonsurans*; Mg, *Microsporium gypseum*; Ep, *Epidermophyton sp.*; Sc, *Saccharomycetes cerevisiae*; CA, *Candida albicans* (Pharmacy Dept Kings College culture collection); CLT, clotrimazole.

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Investigation of some Chinese traditional medicines used to treat cancer

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Five traditional Chinese medicines (TCM), four plants and one animal, were selected for research into their reputed anti-cancer effects. They were *Illicium verum* Hook.f fruits (IV), *Lonicera japonica* Thunb flowers (LJ), *Aristolochia manshuriensis* Kom stem (AM), *Dolichos lablab* L seeds (DL) and *Gekko swinhoana* Gunther entire animal (GG). The selection was made on the basis of their reputation as folk medicines in treatment of tumours and related disorders (YuBin 1999). They are used to treat some kinds of carcinomas or reported to have in-vitro anti-cancer activity (Nam et al 2003). In-vitro cytotoxic screening of extracts made with hexane, chloroform, methanol and water was carried out by using the Sulphorhodamine B (SRB) assay (Lin et al 1999). Three cancer cell lines, COR-L 23 (human non-small cell lung cancer, ECACC no. 92031919), C32 (human amelanotic melanoma, ECACC no. 87090201) and HepG2 (human Caucasian hepatocyte carcinoma, ECACC no. 85011430) were used for the primary screening. The optimal inoculation density for each cell line ensures exponential growth throughout the experimental period and ensures a linear relationship between absorbance and cell number. The ideal plating densities for the current assays were found to be 2×10^3, 5×10^3, and 4×10^3 cells per well for COR-L23, C32 and HepG2 cell lines, respectively. The optical density readings were taken at 492 nm on SpectraMax-190 (Molecular Devices, Sunnydale, USA). Results are shown in Table 1. Chloroform extracts of IV, LJ and AM showed considerable cytotoxicity with IC50 values of 52 $\mu g\,mL^{-1}$, 24 $\mu g\,mL^{-1}$ and 14 $\mu g\,mL^{-1}$, respectively against COR-L 23 (lung cancer cell line) (Table1). Interestingly, the chloroform extract of *Illicium verum* Hook.f, (IV) was selectively cytotoxic for lung cancer (Table 2) and has IC50 > 100 $\mu g\,mL^{-1}$ for MRC-5, a non-cancer human fetal lung fibroblast cell line, ECACC 84101801.

Table 1 IC50 values ($\mu g\,mL^{-1}$) for chloroform extracts in 72 h treatment

Extracts	COR-L23	C32	HepG2
IV	52 ± 2.74	72 ± 1.99	> 100
LJ	24 ± 0.63	> 100	92 ± 1.04
AM	14 ± 2.28	28 ± 1.28	> 100
DL	> 100	> 100	> 100
GG	> 100	> 100	> 100

Table 2 IC50 values ($\mu g\,mL^{-1}$) of IV chloroform extract on cancer and normal cell lines

Cell line	CORL-23	MRC-5
72 h treatment	65.5 ± 4.51	> 100
144 h treatment	48.6 ± 7.28	> 100

Data are expressed as mean ± s.e.m., n = 3.

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Extraction of delphinium pacific giant seeds

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Delphinium and *Consolida* species are highly toxic to cattle, of economic concern across North American ranges (Panter et al 2002). The norditerpenoid alkaloid methyllycaconitine (MLA) is the constituent that contributes a major part of this toxicity and is a most potent, selective, and competitive antagonist for nicotinic acetylcholine receptors. The molecular basis for selective receptor blockade makes MLA and other related norditerpenoid alkaloids lead molecules in this research area (Hardick et al 1996). The basic nitrogen atom in MLA is a part of a substituted *N*-ethylpiperidine moiety. However, the corresponding *N*-ethyl nitrogen atoms in piperidine containing polycyclic norditerpenoid alkaloids are found to be significantly weaker bases than *N*-ethylpiperidine ($pK_a = 10.45$). Thus, substitution with secondary alcohol and/or *O*-methyl ether functional groups acts to reduce the basicity, so that typically observed pK_a values ("apparent" in 50% aq. ethanol, as they are only sparingly aqueous soluble) include: elatine (5.33), condelphine (6.45), neoline (6.70), aconitine (7.23), lycoctonine (7.50) (Golkiewicz et al 1968) and delpheline (7.6). The basicity of inuline (anthranoyl lycoctonine) and MLA are not yet reported, but this physico-chemical parameter will significantly modulate the fractions in which MLA is discovered during the isolation protocol and its distribution in biological systems. Ground Delphinium Pacific Giant seeds (500 g) were extracted in a soxhlet thimble with hexane, dichloromethane and ethanol sequentially (5 cycles each, 2 L scale). After concentration in-vacuo the residues from each extract were extracted with 0.5 M H_2SO_4 (4 × 200 mL). The pH of the combined acidic layers was adjusted to 4.6 with solid $NaHCO_3$, to pH 7.1 with solid Na_2CO_3, and to pH 10.0 with 0.1 M NaOH; each fraction was back extracted with dichloromethane (5 × 100 mL). The combined organic extracts were washed with water (1 × 50 mL), dried ($MgSO_4$), and concentrated in-vacuo yielding 3 fractions (F) 1, 2, and 3, respectively, total alkaloid yields were: F1 (1.5 g), F2 (1.5 g) and F3 (0.5 g) from the hexane extract; F1 (3.5 g), F2 (2.1 g) and F3 (0.6 g) from the dichloromethane extract; F1 (2.4 g), F2 (0.8 g) and F3 (0.4 g) from the ethanol extract. TLC analysis (cyclohexane–chloroform–diethylamine 5:4:1) showed that MLA was present in each fraction of three solvent extracts except F3 of the ethanol extract. Delpheline was present in F1 and F2 of all three solvent extracts. MLA was isolated by column chromatography and its structure established on the basis of ^1H, ^{13}C, DEPT, COSY, HMQC and HMBC NMR spectroscopic techniques. We are now purifying MLA to homogeneity to determine its "apparent" pK_a value.

We acknowledge the Collaborative Research Network (CRN) for a Royal Thai Government studentship (to P.S.).

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Antioxidant activity of *Eclipta alba* in rat liver

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Antioxidants are necessary for preventing the formation of free radicals and the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins. The plant *Eclipta alba* L. Hassk. (Asteraceae) has been reported to show protective effect on experimental liver damage in rats and mice (Singh et al 1993). Further, wedelolactone a major constituent is a potent and selective 5-lipoxygenase inhibitor (Wagner & Fessler 1986). Since the preliminary screening of methanolic extract exhibited significant free radical scavenging and inhibition of lipid peroxidation, this study was planned to further evaluate the antioxidant potential of the ethyl acetate fraction of the aerial parts of the plant to assess the role of a possible antioxidant effect in the hepatoprotective action of the drug. The dried, powdered, aerial parts of the plant were extracted with methanol in a soxhlet. The solvent was removed and the extract was suspended in water and heated on a steam bath (30 min, < 80°C). After filtration, the water phase was partitioned with ethyl acetate. The organic

fraction was dried with sodium sulphate and the solvent evaporated to yield ethyl acetate fraction of *Eclipta alba* (EAEA). The radical scavenging potential of EAEA was assessed for interaction with 1,1-di phenyl, 2-picryl hydrazyl (DPPH$^{\bullet}$) stable free radicals and for inhibition of lipid peroxidation in rat liver homogenate in-vitro as thiobarbituric acid reactive substances (TBARS) at various concentrations (10–400 $\mu g\,mL^{-1}$). EAEA (50, 100 and 200 $mg\,kg^{-1}$) was suspended in 0.3% carboxy methyl cellulose in distilled water and administered orally once daily for 7 days to young adult charles foster rats (180–220 g). The control rats received an equivalent volume of the vehicle orally for the same time periods. Rats were killed 1 h after the last drug or vehicle administration on day 7. Liver were excised, washed in ice cold EDTA-saline and homogenized in 10 volumes of ice-cold 20 mM phosphate buffer (pH 7.0). This was incubated with triton X-100 at a concentration of 0.1% for 1 h at 5°C and centrifuged (20 000 *g*, 20 min) and the supernatants were used for measuring activity of enzymes catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). Protein contents were quantified. The EAEA showed significant ($P < 0.001$) interaction with stable free radical DPPH$^{\cdot}$ expressing scavenging activity in the concentration range of 25–400 $\mu g\,mL^{-1}$ to the highest extent of 83.49%. The extract inhibited the lipid peroxidation induced in the rat liver homogenate significantly ($P < 0.001$) in the concentration range of 10–400 $\mu g\,mL^{-1}$ in a dose-dependent manner. At a concentration of 400 $\mu g\,mL^{-1}$ the extract exhibited 100% inhibition of lipid peroxidation. Further, administration of EAEA to experimental rats at 50, 100 and 200 $mg\,kg^{-1}$ daily for 7 days resulted in a significant elevation in the activity of antioxidant enzymes SOD, CAT, GPx and GR in liver. Thus EAEA exerts antioxidant protection by activating the associated antioxidant enzymes.

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Antiprotozoal activity guided fractionation of *Sclerocarya birrea* (A. Rich.) Hochst

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Malaria is an infectious disease that poses a serious threat to global health, accounting for 2.6% of the total disease burden of the world (WHO's Fact Sheet 1998). Although confined to the tropical and sub-tropical regions of the world, studies suggest that the number of malarial cases may double in over a decade if new methods of control are not devised and implemented (Breman 2001). In developing countries, herbal remedies have been successfully used for centuries for both the treatment and prophylaxis of malaria and are still being used. The Research Initiative on Traditional Antimalarial Methods (RITAM) supports that their use should be scientifically validated and investigated, and this research is in accordance. Previous screening of 7 traditional plants showed that *Sclerocarya birrea* (Anacardiaceae) (Marula tree) had significant and selective in-vitro antiplasmodial and antitrypanosomal activity (Anao et al 2003). *S. birrea* bark is used for the treatment of fevers and malaria in Southern Africa. Hexane, dichloromethane, methanol and water extracts were made of the bark and were tested in-vitro against *Plasmodium falciparum* 3D7 and K1 using the radiolabelled ^{3}H-hypoxanthine assay, *Trypanosoma brucei rhodesiense* STIB900 and mammalian KB cell line using the Alamar Blue Oxidation-Reduction assay. The DCM extract was the most active with IC50 values of 31.1 $\mu g\,mL^{-1}$ against 3D7 (*P. falciparum*), 17.8 $\mu g\,mL^{-1}$ against K1 (*P. falciparum*), 21.2 $\mu g\,mL^{-1}$ against *T. brucei rhodesiense* STIB900 and 79.4 $\mu g\,mL^{-1}$ against KB cells. This extract has been subjected to bioactivity guided fractionation using column chromatography (flash and gravity), preparative thin-layer chromatography and high-pressure liquid chromatography. After each stage of fractionation, resulting smaller fractions have been tested for antiplasmodial and antitrypanosomal activity in-vitro and results obtained so far are encouraging, because the various IC50 values are getting lower, indicating that we are narrowing in on the antiprotozoal active compounds that are responsible for the activity of the crude parent DCM extract. Six fractions were obtained after the separation of the DCM extract by Flash column chromatography while using DCM and methanol in a step gradient elution. Fraction C6, which was the most active, was then subjected to gravity column chromatography eluting with hexane, DCM and methanol mixtures by increasing the polarity to obtain a further 12 fractions. The most active and selective fractions C6/7, C6/8 and C6/9 had IC50 values against 3D7 12.8 $\mu g\,mL^{-1}$, 7.8 $\mu g\,mL^{-1}$ and 6.3 $\mu g\,mL^{-1}$, respectively, and against STIB900 10.1 $\mu g\,mL^{-1}$, 15.1 $\mu g\,mL^{-1}$ and 14.8 $\mu g\,mL^{-1}$, respectively. Further investigation and fractionation are in progress to isolate the active compounds.

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Fungi as a source for novel drugs

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Fungi have been used in diet and traditional medicine for thousands years. The discovery of penicillin in 1928 and subsequently other antimicrobial and antitumour antibiotics has scientifically proved the medicinal importance of fungi. However, although fungal secondary metabolites represent a large portion of the known anti-infective and anti-tumour biologically-active compounds, of the conservatively-estimated 1.5 million fungal species, only approximately 5% have been described, while only 0.3% have been screened for biological activity (Coombes 1992). Exploration of ecologically "unusual" habitats, has led to the discovery of a considerable number of novel and even more diverse microbial species. These untapped microbial "factories" possess immense potential for discovery of novel compounds and biotransformation as source of valuable drugs. A series of novel or untapped fungal species has been explored in this study for their in-vitro cytotoxic activity. Fungal extracts were tested by an in-vitro assay based on the NCI (National Cancer Institute) method for screening for natural products with anti-cancer activity (Skehan et al 1990), against cancerous and normal cell lines (COR-L23 (Human Caucasian lung large cell carcinoma), C32 (Human Amelanotic melanoma), ACHN (Human renal Adenocarcinoma), HK-2 (Human normal kidney cells line), MRC-5 (Human foetal lung, fibroblast-like)). Crude extract ($CHCl_3$:MeOH) of the filamentous fungus coded "**P**" for confidentiality purposes showed the most potent cytotoxic activity against COR-L23 cell line with IC50 5.23 $\mu g\,mL^{-1}$. Vacuum Liquid Chromatography was used as the first step in the bioactivity guided fractionation during which 15 (P1-P15) fractions were obtained. Cytotoxicity was located in the fractions P7-P11, and the most active being P9 IC50 1.51 $\mu g\,mL^{-1}$. Further work is currently in progress to elucidate and identify the active compound(s).

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The anti-staphylococcal activity of *Chamaecyparis* terpenoids

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is frequently mentioned in the media and is described rather alarmingly as a 'superbug' with resistance to all antibiotics except vancomycin. MRSA is now a major problem not only in hospitals, being a major cause of nosocomial infection, but also in the community. New ways of treating MRSA together with new antibacterial compounds that will either kill or prevent the growth of MRSA are urgently required. This project is part of a continuing study to identify anti-staphylococcal compounds from the immature cones of conifers. Here, the cones of *Chamaecyparis lawsoniana* and *C. nootkatensis* were investigated. The cones were exhaustively extracted using a soxhlet, with solvents of increasing polarity. Minimum inhibitory concentration (MIC) assays determined that the hexane and chloroform extracts were active (MIC 8–16 $\mu g\,mL^{-1}$) and the acetone, methanol and water extracts were inactive. Vacuum-liquid chromatography followed by either solid phase extraction or sephadex column chromatography and finally preparative TLC yielded compounds from the active fractions. NMR was used to identify the compounds which so far have been known sesquiterpenes and diterpenes, including ferruginol. These were tested against several multidrug-resistant (MDR) strains of *S. aureus*. Some of the diterpenes showed activity against MRSA with an MIC of 4 $\mu g\,mL^{-1}$ and were bactericidal at 128 $\mu g\,mL^{-1}$. Multidrug efflux pumps are a major cause of

antibiotic resistance in *S. aureus*. Tegos et al (2002) reported that inactivation of MDR pumps can lead to a significant increase in the activity of known antibacterial compounds. Also, that disabling MDR pumps can be used to identify plant antibacterials. The antibacterial and resistance modifying activity of *Chamaecyparis* diterpenes against MDR strains of *S. aureus* will be discussed. Until recently, treatment with one antibiotic was expected to deal with a bacterial infection, but in future a more inclusive approach to such infections will be needed. This may include, for example, using a combination of antibiotics to be taken systemically, together with topical application of antibacterials. Many plant antibacterials are cytotoxic and therefore unsuitable for systemic use. Using an inhibitor of bacterial efflux pumps could lower the concentration at which an antibiotic is effective (Tegos et al 2002; Gibbons et al 2003). Treatment of infections with a combination of antibiotics may help to reduce or prevent resistance in bacteria. It is possible therefore that plant antibacterials may have a future role to play in fighting the threat of MRSA.

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α-Amylase inhibitors extracted from plants traditionally used for diabetes and their potential as novel anti-diabetic treatments

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The endocrine disease diabetes mellitus, characterised by hyperglycemia and arising from a defect in both insulin secretion and insulin resistance, has been known for centuries and many plants are refuted to be useful in its treatment. A recent review (Marles & Farnsworth 1995) showed that 81% of traditional antidiabetic plants screened for diabetes lowered blood-glucose levels, accounting for 1123 species, 725 genera and 183 families. This inspired research into the development of an assay to test for anti-diabetic activity based upon the inhibition of the digestive enzyme pancreatic α-amylase. The development and testing of 30 traditional Indian plants (Bawden et al 2002) yielded a hexane extract of *Murraya koenigii* Spreng. (Rutaceae) and *Cyperus rotundus* L. (Cyperaceae), which showed significant α-amylase inhibitory activity. A sequential soxhlet extraction using hexane, chloroform, methanol and water confirmed that the hexane extract of *Murraya koenigii* ($1\,mg\,mL^{-1}$) and the methanolic extract of *Cyperus rotundus* ($1\,mg\,mL^{-1}$) contained the highest levels of the active inhibitory compounds (44.79, 61.95% inhibition, respectively). Fractionation of the hexane *Murraya koenigii* extract was carried out using techniques including vacuum liquid chromatography, column and flash chromatography using solvent systems of increasing polarity from 100% hexane to 100% methanol. Thin layer chromatography (TLC) identified fractions with similar compounds developed in suitable ratios of hexane:chloroform, sprayed with 10% sulphuric acid and observed under 365 nm "longwave" radiation. These techniques, especially flash chromatography and TLC, yielded fractions A–E, which when tested upon the assay demonstrated varying α-amylase inhibition. Confirmation of the purity, using TLC, of fractions A and B in particular showed a number of impurities and therefore further fractionation based upon them ceased and the whole fractionation procedure was repeated starting with the soxhlet extraction using an increased quantity of plant material. Further assay-guided fractionation is continuing to isolate and characterise compounds with α-amylase inhibition and NMR and mass spectrometry is being employed for characterisation. Fractionation of the methanolic extract of *Cyperus rotundus* using similar techniques has provided fractions showing α-amylase inhibition but further investigations are planned to discover the source of α-amylase inhibitory activity.

This work is supported by a grant from Merck Research Laboratories, Rahway NJ

Table 1 % Inhibition and the yields obtained from fractions A–E

Fraction	Yield/mg	% Inhibition
A	40	54.37
B	20	88.15
C	5	45.78
D	7	15.79
E	6	66.93

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Pyrrolizidine alkaloids of a medicinal plant of Iran: *Echium amoenum* Fisch. & Mey

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Echium amoenum Fisch. & C.A. Mey. (Boraginaceae) is an endemic Iranian plant and, its dry violet-lue petals have long been used as a tonic, tranquillizer, diaphoretic, a remedy for cough, sore throat and pneumonia in traditional medicine of Iran (Hooper 1937). Pyrrolizidine alkaloids have been isolated from Boraginaceae including *Echium* genus (Mattocks 1986). The chemistry of these alkaloids in relation to their toxic and therapeutic effects has been the subject of several investigations (Mattocks 1986; WHO 1988). Because the decoction of *E. amoenum* dry petals is used in folk medicine, we tried to identify the pyrrolizidine alkaloids of its petals. Literature review showed that, except for a brief report, no detailed phytochemical studies on these species have been reported and this is the first report on the isolation of pyrrolizidine alkaloids from this species (Delorme et al 1977). Petals of *E. amoenum* were collected from a farm at 80 km north of Ghazvin in June 2000. Powdered plant material (7 kg) was extracted by soxhlet under reduced pressure by methanol and the extract was evaporated under reduced pressure below 50°C. The concentrated extract was partitioned between ethyl acetate and 2 N HCl in water, acidic layer after addition of Zn dust was stirred for 48 h. Then pH of this aqueous phase was adjusted to 10 and extracted with $CHCl_3$ and concentrated, the crude alkaloids (0.1 g) was separated on silica gel GF_{254} developed by CH_2Cl_2:MeOH: $NH_4OH_{conc.}$ (85:15:2). Two pyrrolizidine alkaloids was purified and the structures elucidated by IR, MS, ^{13}C and ^{1}H NMR and two-dimensional NMR. Echimidine (10 mg) and 7-angeloyl retronecine (6 mg) were isolated from *Echium amoenum* petals (7 kg). The structures of all compounds were confirmed by spectroscopic methods. These two alkaloids are common in *Echium*, but petals of *E. amoenum* have low amounts. Also *E. vulgare, E. rauwolfi*, *E. setosum and E. horridum* have echimidine and 7-angeloyl retronecine (Man'ko 1964; El-Shazly et al 1996a, 1999). Echimidine was reported in *E. humile, E. pininana* and *E. plantagineum* (Culvenor et al 1981; El-Shazly et al 1996b; Reoder et al 1991).

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Estimation of nickel and cobalt in herbal products

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Alternative systems of medicine, like herbal, acupuncture aromatherapy, naturopathy, homeopathy, etc., are nowadays gaining point of interest because of lesser side effects and acceptability to majority of the population. Some managed care organizations now offer these therapies as an expanded benefit. Because the safety and efficacy of these practices remain largely unknown, there is an urgent need to establish the identity, purity and quality assurance of herbal drugs and other preparations used in alternative systems of medicine to ensure the full efficacy and safety of the herbal product. In this study accumu-

lation of nickel and cobalt in the herbal preparations of India viz. *Churna, Gugulu, Bati, Ras, Pishti* and *Arishtas* was determined. The total numbers of 11 different types of marketed products were taken for the estimation of nickel and cobalt. Samples were prepared by Wet Ashing. An accurately weighed 1 g of the sample was dried, grounded and soaked in 10 mL of nitric acid. Samples were then treated with 3 mL of 60% perchloric acid and heated cautiously until nitric acid was evaporated. Solutions were cooled and diluted with 20% hydrochloric acid to 50 mL. Samples so obtained were analysed for the contents of nickel and cobalt by Atomic Absorption Spectrophotometry (GBC – AVANTA 932 plus). Results shown in Table 1 indicate the varying amount of nickel and cobalt in these herbal preparations. These metal ions may have entered through the raw material or during processing. However, some herbal remedies do contain metal ions as their therapeutically active ingredients. So herbal remedies should be consumed with great caution, only under the supervision of a practitioner of traditional medicine. We should not consider them as dietary supplements so as to have complete benefits of these preparations.

Table 1 Concentration of nickel and cobalt in herbal remedies

Formulation	Ni ($\mu g\,g^{-1}$)	Co ($\mu g\,g^{-1}$)
Triphala Churna	29.657	97.774
Sitopaladi Churna	21.342	64.593
Yogaraj Guggulu	25.667	11.494
Trayodashang Guggulu	26.959	28.738
Rajahprawartini Bati	22.559	77.690
Prabhakar Bati	27.941	60.510
Kamdudha Ras	26.212	17.660
Jaharmohra Khatai Pishti	28.579	43.582
Muktashuki Pishti	28.582	215.438
Ashokarishta	19.939	81.021